Response properties of isolated mouse olfactory receptor cells

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1. Response properties of isolated mouse olfactory receptor cells were investigated using the suction pipette technique. Cells were exposed to the odour cineole or to solutions of modified ionic content by rapidly changing the solution superfusing the cilia. All experiments were performed at 37 °C.

2. Mouse olfactory receptor cells displayed a steep dependence of action potential frequency on stimulus concentration, a 3-fold increase in stimulus concentration often saturating the firing frequency at 200–300 Hz. The receptor current increased more gradually with increasing cineole concentration and did not saturate within the 100-fold range of cineole concentrations applied.

3. When stimulated for 30 s with a low odour concentration, cells responded with sporadic spike firing. Higher concentrations led to the generation of a large receptor current at the onset of stimulation which returned to baseline levels within a few seconds, accompanied during its rising phase by a short burst of action potentials. Thereafter an oscillating response pattern was observed during the remainder of the stimulus, consisting of repetitive increases in receptor current of around 1 s duration accompanied by short bursts of action potentials.

4. Olfactory adaptation was studied by comparing the responses to two closely spaced odour stimuli. The response to the second odour stimulus recovered to 80% of its original magnitude when the cell was superfused with Ringer solution during the 5 s interval between odour exposures. In contrast, exposure to a choline-substituted low Na^+ solution between odour stimuli had two effects. First, the receptor current response to the first odour stimulus did not terminate as quickly as in the presence of Na^+, suggesting the presence of a Na^+–Ca^{2+} exchanger. Second, the response to the second stimulus only recovered to 55% of its original magnitude, demonstrating the involvement of Na^+–Ca^{2+} exchange in the recovery of sensitivity in mouse olfactory receptor cells following stimulation.

A wide variety of electrophysiological techniques have successfully been applied to record odour-induced single unit activity from amphibian olfactory receptor cells (Shibuya & Shibuya, 1963; Døving, 1964; Trotier & MacLeod, 1983; Firestein & Werblin, 1989; Kurahashi, 1989; Frings & Lindemann, 1990; Lowe & Gold, 1991; Firestein *et al.* 1993; Reisert & Matthews, 1999*a*) and a vast body of knowledge has accumulated describing their responses to odour stimulation. In general, stimulation leads to the generation of a depolarising receptor current which triggers spike firing of increasing frequency with increasing odour concentration.

In contrast, electrophysiological recordings from isolated mammalian olfactory receptor cells have been difficult to achieve due, in large part, to the small size and fragility of these cells. These problems are compounded further by the need to deliver well-defined odour stimuli in solutions maintained at mammalian body temperature. A variety of approaches have been utilised to address these problems. Extracellular spike recordings from individual receptor cells in a whole-head preparation in mice and rats (Gesteland & Sigwart, 1977; Sicard, 1986; Duchamp-Viret *et al.* 1999) have revealed that increasing stimulus concentrations lead to the generation of spike trains of increasing frequency. Those few electrical recordings that have been made from isolated mammalian olfactory receptor cells reveal that they respond to odour exposure with an inward current (Maue & Dionne, 1987; Lowe & Gold, 1993, 1995; Chiu *et al.* 1997), which is mainly carried by an excitatory Ca^{2+}-activated Cl^- conductance (Lowe & Gold, 1993). However, until the very recent application of whole-cell or perforated patch recording techniques to an intact olfactory epithelium preparation (Ma *et al.* 1999) only very limited information was available as to how this response depended on odour concentration. In the present study we chose instead the suction pipette technique (Baylor *et al.* 1979; Lowe &

Gold, 1991; Reisert & Matthews, 1998) to record from isolated mouse olfactory receptor cells. This non-invasive technique not only provides stable recordings over an extended period at body temperature but also allows the simultaneous recording of the odour-induced spike firing and receptor current responses (Reisert & Matthews, 1999*a*).

Ca^{2+} is known to play a pivotal role in olfactory transduction (for a recent review see Menini, 1999). During the odour response, Ca^{2+}, which enters the cell through cyclic nucleotide-gated channels (Frings *et al.* 1995; Leinders-Zufall *et al.* 1997), activates an excitatory Ca^{2+}-activated Cl^- conductance thereby increasing the magnitude of the total receptor current (Kleene & Gesteland, 1991; Kurahashi & Yau, 1993; Lowe & Gold, 1993; Zhainazarov & Ache, 1995). Furthermore, Ca^{2+} also has profound negative feedback effects on various stages of olfactory signal transduction including the cyclic nucleotide-gated channel, adenylyl cyclase and phosphodiesterase (Borisy *et al.* 1992; Kramer & Siegelbaum, 1992; Chen & Yau, 1994; Yan *et al.* 1995; Wei *et al.* 1996, 1998; Kurahashi & Menini, 1997; Leinders-Zufall *et al.* 1999). Therefore control of intracellular Ca^{2+} levels is crucial in olfactory receptor cells not only for determining response duration but also in the process of adaptation. While the Ca^{2+} influx pathway is quite well understood, little is known about Ca^{2+} removal from the intraciliary space in rodents. In frog olfactory receptors Ca^{2+} is believed to be extruded across the ciliary membrane by a Na^+–Ca^{2+} exchanger (Reisert & Matthews, 1998). In rat olfactory epithelium the presence of a Na^+–Ca^{2+} exchanger in the soma, dendrite and possibly in the cilia has been reported (Noe *et al.* 1997) but its functional significance is unclear. We therefore used multiple rapid solution changes to investigate the functional presence of a Na^+–Ca^{2+} exchanger in mouse olfactory receptor cells and its contribution to response recovery. Preliminary results from this study have been presented to The Physiological Society (Reisert & Matthews, 1999*b*, 2000).

METHODS

Preparation

Male mice (C57BL/6/Ole/Hsd; Harlan Ltd, Bicester, UK), 5–10 weeks old, were killed by cervical dislocation, decapitated and the head split along the septum. The turbinates with the olfactory epithelia were removed and stored in oxygenated mammalian Ringer solution at 4°C until required. In early experiments the epithelia were digested in 0.4 mg ml^{-1} trypsin in mammalian Ringer solution for 30 min, and the enzyme was then quenched with 0.1% trypsin inhibitor (both from Sigma). In the majority of experiments the epithelium from a single turbinate was briefly vortexed in an Eppendorf tube containing 200 μl mammalian Ringer solution and no enzymes. Cells were allowed to settle for 30 min in the recording chamber, which was mounted on an inverted microscope with phase contrast optics (TMS, Nikon, Kingston, UK). Cells were identified by their characteristic bipolar shape; sometimes short cilia were visible at the dendritic knob.

Electrical recording

The suction pipette technique was used to record from isolated mouse olfactory receptor cells (Baylor *et al.* 1979; Lowe & Gold, 1991; Reisert & Matthews, 1998). The cell body of an isolated olfactory receptor cell was drawn into the tip of a suction pipette, leaving the dendrite and cilia exposed to the bathing solution. Recordings could last up to 1 h until unacceptable rundown of the odour-induced response occurred or the cell was lost due to slippage in the tip of the recording pipette. The odour-induced suction pipette current was recorded with a patch clamp amplifier (Warner PC501, Warner Instruments, Hamden, CT, USA). At the time of the experiment the suction pipette current signal was filtered over a bandwidth of DC to 20 Hz (8-pole Bessel filter), digitized at 100 Hz using an intelligent interface card (Cambridge Research Systems, Rochester, UK) and stored on the hard disk of an IBM-compatible computer. The signal was also recorded for subsequent analysis at a wide bandwidth on a modified digital audio tape recorder (DTC-1000, Sony, Tokyo, Japan) and later filtered over a bandwidth of DC to 5 kHz and resampled at 10 kHz to resolve the action currents underlying individual action potentials.

External solutions, solution changes and temperature control

Mammalian Ringer solution contained (mM): NaCl 140, KCl 5, $MgCl_2$ 1, $CaCl_2$ 2, EDTA 0.01, Hepes 10 and glucose 10, adjusted to pH 7.5 with NaOH. Odour solutions were made fresh every second day from a 1 mM cineole stock by a single dilution. The low Na^+–choline solution contained 140 mM choline chloride instead of NaCl. Rapid solution changes were achieved by stepping the interface between neighbouring streams of solution across the tip of the suction pipette. A more detailed description of the superfusion system can be found in Reisert & Matthews (1999*a*). Stepping into a solution of different ionic composition resulted in a junction current between pipette and bath. This was corrected by subtracting the junction current evoked by the same solution change in the absence of odour stimulation, obtained within 10–20 s of the actual recording. The junction current subtraction was sometimes imperfect, due to slight variations in its magnitude or time course between presentations, resulting in fast artefactual transients at the moment of solution change. The solution change was typically complete in around 70 ms, as assessed from the rise of the junction current. The times of application and removal of the odour stimulus have been corrected according to the half-rise time of the junction current.

All experiments were performed at 37°C. Each stream of solution flowing into the recording chamber passed through an electrically and thermally insulated heating unit consisting of a 19 gauge stainless steel tube in thermal contact with a 4 W wire-wound resistor in a ceramic housing. The temperature of the heater was monitored with a thermocouple and controlled by a digital temperature controller (Type 9900, CAL controls, Hitchin, UK). A more detailed description of the flow heater can be found in Matthews (1999). The small heat loss which occurred as the solution passed between the heater and the recording chamber was compensated by measuring the temperature in the chamber with a second thermocouple and increasing the temperature of the heater accordingly.

Data analysis

Instantaneous spike frequency was calculated as the reciprocal of the shortest time interval between neighbouring pairs of spikes within a given sweep and the maximal spike frequency within each burst was used to derive the dose–response relationship for spike firing. The period of the current oscillations that were observed during prolonged odour stimulation was determined by measuring the time

after stimulus onset of each elevation in receptor current, calculating the time differences between neighbouring pairs of current maxima within a given sweep, and averaging these time differences. The time interval between the first and the second peaks was excluded from the average since the receptor current declined more slowly from its first than from subsequent maxima.

RESULTS

Responses to brief odour stimulation

The dose dependence of spike firing and receptor current responses were investigated by stimulating an isolated mouse olfactory receptor cell with a 1 s exposure to the odour cineole over a range of concentrations (Fig. 1). The lowest concentration of 10 μM evoked no response whatsoever, while an increased concentration of 30 μM elicited a large receptor current accompanied during its rising phase by a short burst of action currents at high frequency (see Fig. 1, right-hand panels). The amplitude of successive action currents decreased progressively within the burst (see also Fig. 3, inset). Increasing the stimulus concentration further resulted in a progressive increase in receptor current. At the highest concentrations the waveform of the receptor current developed an initial peak followed by a maintained plateau phase (see also Fig. 4) which could outlast the end of stimulation by several seconds.

The frequency of spike firing is plotted for this cell in Fig. 2*A* as a function of stimulus concentration. Spike firing frequency varied steeply with odour concentration, rising from firing threshold to a near-saturated level for a 3-fold increase in concentration. The dose–response relationship for spike firing was investigated in a total of 10 cells. In three of these cells, the dose–response relationship for spike firing rose steeply with increasing odour concentration; stimulus concentrations at or below a critical value did not elicit a response, while exposure to the next-highest available concentration (an approximately 3-fold increase) was sufficient to drive the firing frequency close to saturation. In these three cells the spike rate elicited by the lowest concentration that evoked a response reached 76–86% of its saturated value (taken as the average firing frequency elicited by the two highest concentrations applied). In a fourth cell, increasing the odour concentration elicited a comparably abrupt elevation in firing frequency above the sporadic firing evoked by the next-lowest odour concentration, which amounted to 15% of the saturated value.

In contrast, in three further cells, the firing frequency appeared to increase in a more gradual manner with odour concentration when averaged across repeated trials. However, we believe that this more graded variation in

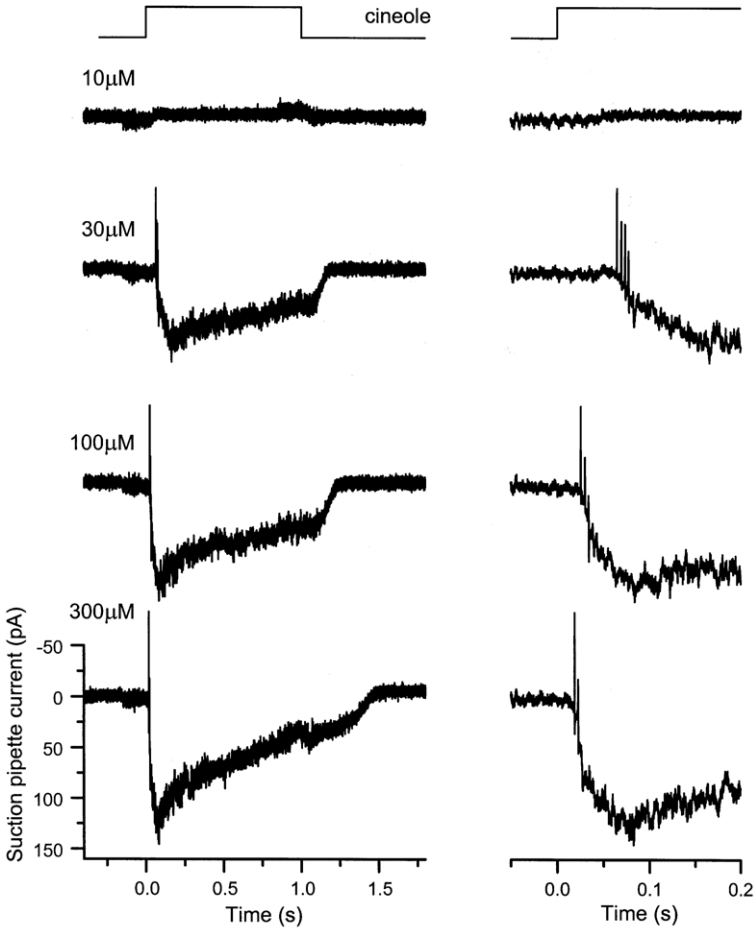

Figure 1. Odour-induced responses from an isolated mouse olfactory receptor cell recorded with the suction pipette technique

An isolated olfactory receptor cell was exposed for 1 s to the odour cineole by rapidly translating the interface between neighbouring streams of solution across the cell. The applied concentrations ranged from 10 to 300 μM, as indicated beside each trace. The suction pipette current was recorded over a wide bandwidth (DC to 5000 Hz). Upper traces represent the timing of the solution change. The experiment was carried out at 37°C. Right-hand panels show the rising phase of the response to the odour stimulus on an expanded time base.

firing frequency as a function of odour concentration may have been more apparent than real. In these cells, high odour concentrations evoked a large receptor current response with an accompanying high frequency burst of action potentials at the onset of stimulation, much as illustrated in Fig. 1. In contrast, lower odour concentrations elicited a barely discernible receptor current which evoked multiple short bursts of action potentials at a high frequency (100 Hz or greater) in some trials, while in others the cell remained silent altogether. Consequently, when the peak firing frequency was averaged across multiple trials an artefactual intermediate firing frequency was calculated, which was never actually exhibited by the cell. We therefore believe that the underlying dose–response relationship in these cells is likely to have been much steeper than that estimated from these averaged responses. In the remaining three cells the range of available odour concentrations was unfortunately too high to allow the spike frequency to fall significantly below its maximal value, so no dose–response relationships could be constructed.

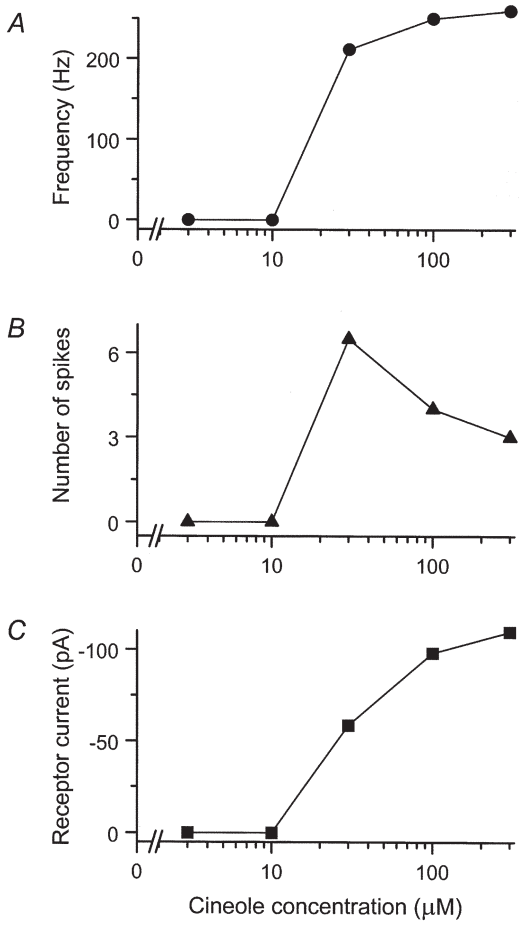

Figure 2. Dose–response relationships for receptor current and spike firing

Spike frequency (*A*), number of spikes fired (*B*) and peak receptor current (*C*) plotted as a function of the cineole concentration. The data were derived from the cell of Fig 1; each data point represents the average of two trials.

The maximal firing frequency evoked by high odour concentrations ranged from 230 to 300 Hz in different cells. The number of spikes fired in response to the odour stimulus first rose with increasing concentration (Fig. 2*B*) but then progressively declined to as few as two spikes at the highest concentrations. The maximum number of spikes fired per stimulus by a given cell was relatively low, varying between four and nine spikes (average of 6 spikes fired, 7 cells tested). Extended spike trains were never observed in response to a 1 s odour exposure, the period of spike firing always terminating before the end of the stimulus.

The peak receptor current (Fig. 2*C*) increased rather more gradually with increasing stimulus concentration than the frequency of spike firing. Moreover, the available concentration range of 3–300 μM cineole was not sufficient to saturate the receptor current completely in the 10 cells tested. Nevertheless, the peak receptor currents evoked by the highest cineole concentrations could attain −400 pA and reach their peak within 100 ms after the onset of the stimulus. Although the failure to saturate the receptor current prevents the unequivocal determination of the range of concentrations over which the transduction mechanism can operate, it seems clear that the dynamic range of the receptor current response is considerably greater than that for spike firing in isolated mouse olfactory receptor cells.

Responses to prolonged odour stimulation

In order to investigate how the continuous presence of odour is encoded, isolated mouse olfactory receptor cells were stimulated with cineole for 30–60 s. At the lowest concentration of 10 μM (Fig. 3, 30 s stimulation), sporadic spike firing was observed throughout the period of stimulation. A higher cineole concentration of 30 μM evoked a short burst of action potentials during the rising phase of the now-prominent increase in receptor current at the onset of stimulation. Within approximately 2 s the receptor current decayed to around 20% of its peak value, but thereafter rose and fell periodically, each elevation in receptor current lasting about 1 s. Around 10 s after the onset of stimulation the receptor current declined to near-zero levels between these repetitive responses, and short bursts of action potentials were fired once again on the rising phase of each subsequent elevation in receptor current. This can be seen more clearly in the inset to Fig. 3, which depicts the last two current peaks evoked by 30 μM cineole stimulation. When the cineole concentration was increased further to 100 μM the receptor current declined more gradually after its initial rise at the onset of stimulation, decaying over a period of approximately 10 s. After this time the receptor current began to oscillate for the remaining 20 s of odour stimulation. However, at this higher concentration, no action potentials were evoked by these transient

increases in receptor current. In this cell, the oscillation period evoked by 100 μM cineole was 0.82 s, compared to 0.91 s at 30 μM. Finally, at 300 μM the receptor current did not return to baseline levels during the period of stimulation, no oscillatory response was observed, and action potentials were only evoked at the onset of stimulation.

A total of 13 cells were exposed to cineole for periods of 30 or 60 s. Of these cells, 11 clearly showed oscillatory responses with periods ranging from 0.37 to 2.37 s with a mean value of 1.4 ± 0.2 s (mean $\pm$ S.E.M.) at the lowest concentration tested that evoked an oscillatory response in each cell. In six of these cells several odour concentrations were employed, allowing the dependence of the oscillation period on odour concentration to be examined. This comparison revealed that a 2- to 3-fold increase in stimulus concentration reduced the oscillation period by between 10 and 50% in individual cells. The two remaining cells, which were exposed to cineole for 30 s, did not generate a regular oscillatory response pattern, but instead exhibited sporadic increases in receptor current at irregular times during the odour exposure.

Response termination and the control of sensitivity

The role played by extracellular Na^+ in the processes of response termination and the control of sensitivity were investigated in the experiment of Fig. 4. When the cell was stimulated for 1 s with the high cineole concentration of 300 μM (Fig. 4, thin trace) the response rose rapidly and then relaxed to a plateau level. Once the period of stimulation ceased the receptor current declined rapidly, falling to 50% of its value at the end of stimulation in 0.4 s. If, instead, the cell was exposed to a choline-substituted low Na^+ solution after the end of stimulation, the receptor current was prolonged (thick trace), falling to 50% of its value at the end of stimulation over the next 0.9 s. This corresponds to more than a doubling of the time for this decay in comparison with control conditions when recovery took place in Ringer solution. No abrupt changes in current were seen in this junction-corrected trace upon the step from Na^+-containing to choline-

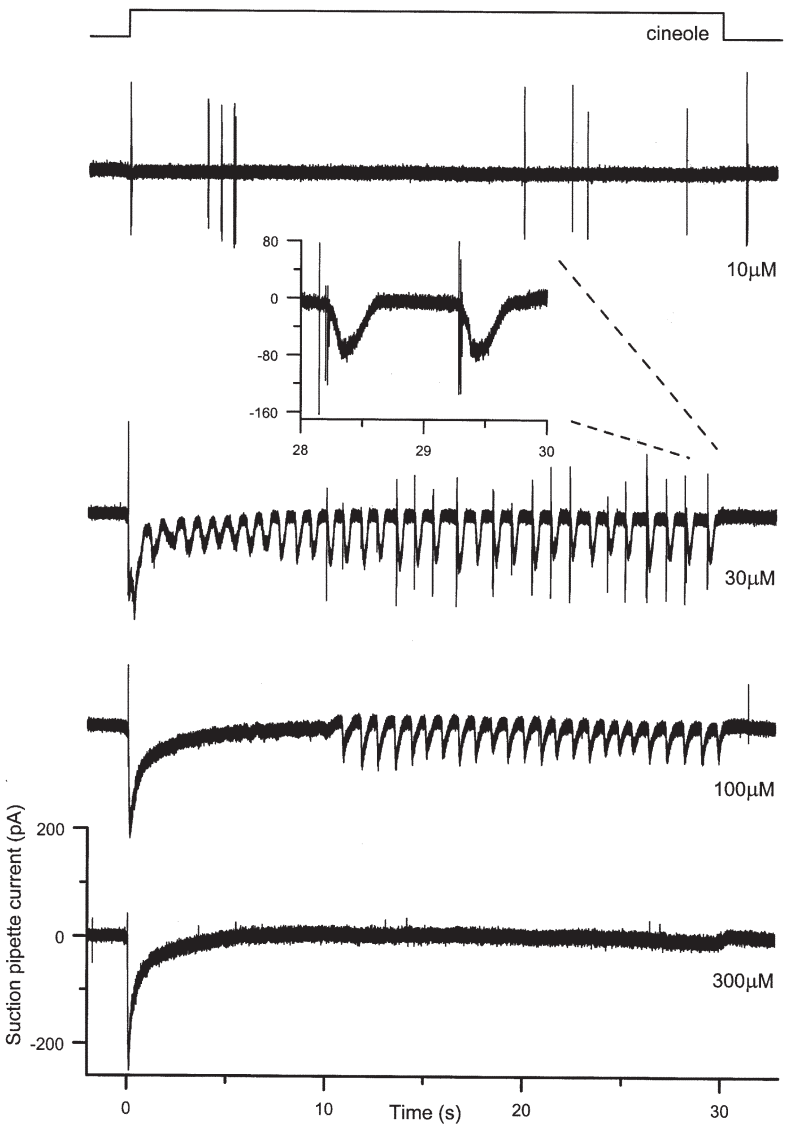

Figure 3. Responses of a mouse olfactory receptor cell to 30 s odour stimulation

An isolated olfactory receptor cell was stimulated for 30 s with the odour cineole at the concentrations indicated next to each trace. The upper trace represents the timing of the solution change. Recording bandwidth, DC to 5000 Hz. A slight DC shift occurred during the recording of the 300 μM trace. Inset, magnified version of the last two oscillatory elevations of current from the 30 μM trace.

substituted solution, suggesting that little current was carried by Na^+ at this time. The prolongation of the receptor current by exposure to choline-substituted solution was investigated in a total of five cells stimulated with a 1 s exposure to 300 μM cineole, the response duration increasing by 0.36 ± 0.10 s (mean $\pm$ S.E.M.) in comparison with the control in Ringer solution. This prolongation corresponds to an increase in the duration of the response following the cessation of stimulation by a factor of 3.3 ± 1.2 in the absence of external Na^+. Similar results were obtained in a sixth cell, which was only tested at 100 μM cineole. In contrast, exposure to low Na^+–choline solution just before odour stimulation did not affect the subsequent response.

Recovery from the adaptation induced by the first odour exposure was investigated by applying a second identical odour stimulus after a recovery period of 5 s. When this recovery took place in Ringer solution, the second stimulus evoked a response of 75% of the original magnitude (Fig. 4, thin trace). But if the cell was exposed to a choline-substituted low Na^+ solution during the recovery period instead of normal Ringer solution, sensitivity recovered to a lesser degree during this interval, and the magnitude of the response to the second stimulus in this cell only reached 54% of that to the first (Fig. 4, thick trace). On average, the response to the second stimulus was $81 \pm 3\%$ (mean $\pm$ S.E.M., 5 cells, 300 μM cineole) of the response to the first after a 5 s recovery period in Ringer solution, while exposure to low Na^+–choline solution during the interval between the two stimuli reduced the extent of recovery to $56 \pm 5\%$; these values are significantly different at the 5% level (Student's paired t test). It would therefore appear that external Na^+ not only influences the kinetics of response recovery, but also speeds the restoration of sensitivity following odour stimulation.

DISCUSSION

Dose–response relationships for mouse olfactory receptor cells

The technical difficulty of stimulating and recording from mammalian olfactory receptor cells at mammalian body temperature has hitherto limited most electrophysiological studies to the larger and more robust olfactory receptor cells of amphibians (Kurahashi & Shibuya, 1990; Firestein & Werblin, 1989; Lowe & Gold, 1991; Firestein *et al.* 1993; Kurahashi & Menini, 1997; Leinders-Zufall *et al.* 1999; Reisert & Matthews, 1999*a*). Those few studies that have recorded single unit discharges from mouse or rat olfactory receptors at body temperature have been carried out in the intact olfactory epithelium *in situ* using relatively brief (2 s) air-borne odour stimuli (Sicard, 1986; Duchamp-Viret *et al.* 1999; Duchamp-Viret *et al.* 2000). Our recordings from isolated mouse olfactory receptor cells have for the first time allowed a direct comparison of these spiking discharges with the receptor currents which gave rise to them in response to well-defined stimuli of both short and long duration. This approach could be used in future to record reliably the responses of isolated olfactory receptor cells of mice carrying transgenes for specific olfactory receptor molecules (Zhao *et al.* 1998) or genetic modifications to individual components of the olfactory transduction cascade (Brunet *et al.* 1996; Belluscio *et al.* 1998).

When studying the dependence of the responses of isolated mouse olfactory receptor cells on stimulus concentration, we were faced with a dilemma. The number of different solutions that could realistically be applied to any given cell was restricted by the finite duration of each recording. In order to balance the conflicting demands of precision with the need to stimulate cells with different sensitivities over a reasonably wide concentration range, we adopted a

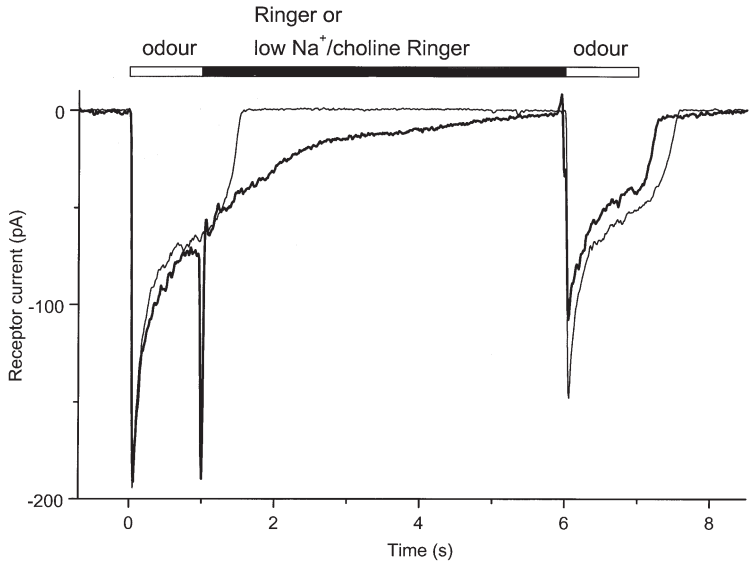

Figure 4. Response prolongation and reduced recovery from adaptation on the removal of external Na^+

A mouse olfactory receptor cell was stimulated twice in succession with a 1 s exposure to 300 μM cineole as indicated by the solution monitor (horizontal bar). During the 5 s recovery interval between the two stimuli the cell was exposed to either normal Ringer solution (thin trace) or low Na^+–choline solution (thick trace). The recording in low Na^+–choline solution has been corrected by subtraction for the junction current. The trace recorded in Ringer solution represents the average of two trials, one recorded before and one after the low Na^+–choline exposure. Currents were sampled at 100 Hz and filtered over a bandwidth of DC to 20 Hz so as to display only the receptor current.

1–3–10 concentration sequence corresponding to increases of around 3-fold in odour concentration. This concentration spacing had previously proved sufficient to map the dose–response relationship for spike firing in frog olfactory receptor cells (Reisert & Matthews, 1999*a*), revealing a Hill coefficient of 1.8 in that species. However, in the four mouse olfactory receptor cells whose dose–response relationships could be characterised in detail in the present study, a much more abrupt rise in the rate of spike firing was observed with increasing stimulus concentration, which could not be resolved fully with this concentration spacing. This observation suggests that the dose–response relationship for spike firing can be even steeper in mammals than in the amphibian. While earlier studies did not attempt to construct detailed dose–response relationships for spike firing, our results contrast with the very recent observation that the firing rate of rat olfactory receptor cells rises *in situ* only as a rather shallow function of air-borne odour concentration, in a similar manner to the frog (Duchamp-Viret *et al.* 2000). While it is conceivable that this might represent a species difference between rat and mouse, it seems more likely that it may, instead, originate from the different method of stimulus presentation employed in the two studies. In our experiments, both the onset and removal of the odour stimulus will have been determined by the rapid exchange of the solution bathing the exposed cilia, which was complete in around 70 ms (see Methods). In contrast, both the rise and subsequent decline in odour concentration in the mucus film overlaying the olfactory epithelium *in situ* may have been much more gradual following the presentation of the air-borne stimulus, a notion supported by the prolonged spike trains observed by Duchamp-Viret and her colleagues in response to nominally brief odour stimuli (Duchamp-Viret *et al.* 2000).

In contrast to the narrow dynamic range which we observed for the spiking responses of isolated mouse olfactory receptor cells, the receptor current recorded by the suction pipette varied over a considerably wider range of odour concentrations. The 100-fold range of odour concentrations used in this study did not allow us reliably to saturate the mouse suction pipette current response, a result which contrasts with the steeper concentration dependence of the whole-cell voltage clamp receptor current observed previously in mouse (Ma *et al.* 1999). Nevertheless, the progressive changes which we observed with increasing concentration in the waveform of the receptor current recorded by the suction pipette were similar in frog and mouse, the principal difference being the faster mammalian response kinetics. In the frog the response latency and time to peak could be readily determined since these were both long in comparison with the rise in odour concentration during stimulation inferred from the time course of the solution change (Reisert & Matthews, 1999*a*). In contrast, mouse olfactory receptor cells responded so rapidly (within a few tens of milliseconds of the nominal completion of the 70 ms solution change) that a reliable evaluation of the response latency as a function of odour concentration could not be performed.

Examination of the rising phase of the odour response reveals that mouse olfactory receptor cells can generate a suction pipette current at the response peak of up to 100 times larger than that required earlier in the rising phase to evoke the maximal spike frequency. Indeed, under voltage clamp, the whole-cell current evoked by odour stimulation can reach 1.5 nA (Ma *et al.* 1999). This situation is broadly analogous to that in frog olfactory receptor cells, which we have shown previously to respond with an increase in suction pipette current over a range of odour concentrations in excess of 300-fold, and to fire only a brief burst of spikes early in the rising phase of the response to a high odour concentration (Reisert & Matthews, 1999*a*). This substantial mismatch between the dynamic ranges of the spike firing and receptor current responses seems likely to originate in part from the high input resistance of mouse olfactory receptor cells. This has the consequence that the opening of even a single ionic channel can depolarise the cell sufficiently to reach action potential threshold and trigger spike firing (Maue & Dionne, 1987; Lynch & Barry, 1989). When combined with the ability of the Ca^{2+}-activated Cl^- conductance to boost the response supralinearly (Lowe & Gold, 1993), it seems possible that even a low odour concentration might evoke sufficient receptor current to depolarize the cell strongly and give rise to a high frequency burst of action potentials. This notion is supported by our observation that in some cells repeated exposure to a low odour concentration elicited either a high frequency burst of action potentials or no response whatsoever, and that firing at intermediate frequencies was not observed. This tendency towards burst firing is accentuated by the progressive collapse in spike amplitude that accompanies suction pipette currents of just a few picoamps (see e.g. Fig. 3, inset), an effect which most probably results from a progressive and ultimately complete inactivation of voltage-gated Na^+ channels (Trotier, 1994). In addition, the presence of a rapidly inactivating K^+ conductance that recovers only slowly from inactivation has been suggested to contribute to the generation of short bursts of spike firing (Lynch & Barry, 1991).

Oscillatory responses to prolonged stimulation

We found that a characteristic feature of the responses of mouse olfactory receptor cells to prolonged stimulation was a regular oscillatory rise and fall in receptor current, accompanied by bursts of spike firing. We have also observed such oscillatory responses in isolated frog olfactory receptor cells, although with a considerably longer oscillation period of around 6 s (Reisert & Matthews, 1997; J. Reisert & H. R. Matthews, unpublished observations). Although previous studies have not

attempted to present prolonged odour stimuli in the mammal, hints of similar behaviour can be seen in the recordings of Sicard (1986), which show the firing of multiple bursts of action potentials that outlast the nominal duration of the stimulus (Fig. 2, trace A in Sicard, 1986). One might speculate that in the whole-head preparation used by Sicard the odour concentration may have fallen only gradually following the nominal end of stimulation, resulting in a stimulus of considerably longer duration than was intended.

Two aspects of the way isolated mouse olfactory receptor cells encode the presence of odour have potentially important implications for subsequent processing within the olfactory bulb. First, the steep dependence of action potential firing on stimulus concentration would not allow for the odour concentration to be reliably encoded over a wide range of concentrations by any single cell. Instead, the concentration of the stimulus seems likely to be encoded in the pattern of afferent activity in a population of olfactory receptor cells with differing absolute sensitivities. Second, mouse olfactory receptor cells responded to both short and long duration odour stimuli with only a relatively brief initial spike train. Hence, although the time of onset of odour stimulation is faithfully encoded, determining the subsequent time course of the odour exposure will depend upon central analysis of the complex bursting response pattern that accompanies continued stimulation. Interestingly the subset of mouse olfactory receptor cells that express a particular olfactory receptor protein is believed to project to only a pair of glomeruli in the olfactory bulb (Ressler *et al.* 1994; Vassar *et al.* 1994; Mombaerts *et al.* 1996), while any individual glomerulus receives input from around 1000 olfactory receptor cells. So despite the limited information that might be contained within the discharge pattern of any individual olfactory receptor cell, the massive degree of convergence at the level of the olfactory glomerulus may improve the situation considerably, and short spike trains might actually be beneficial for encoding the time of arrival of the stimulus. Unfortunately, relatively little is known about the way a glomerulus integrates (or possibly coincidence-detects) afferent spike discharge, or whether the population of olfactory receptor cells that express a given receptor protein exhibits a range of absolute sensitivities.

Functional roles of Na^+–Ca^{2+} exchange in mouse olfactory cilia

When mouse olfactory receptor cells were exposed to a low Na^+ solution after stimulation, the receptor current response was prolonged (Fig. 4). However, no abrupt decrease in current was observed at the moment of the solution change from Ringer to low Na^+–choline solution, suggesting that Na^+ was not the main charge carrier of this prolonged current since choline permeates the mammalian cyclic nucleotide-gated channel only poorly (Balasubramanian *et al.* 1995). The only other ion that would be capable of carrying a large inward current under these conditions is Cl^- (Zhainazarov & Ache, 1995; Reuter *et al.* 1998), passing through the Ca^{2+}-activated Cl^- channel, which can remain open for an extended period if the Ca^{2+} concentration is elevated (Kleene, 1993; Hallani *et al.* 1998). The prolonged elevation of current therefore suggests that in the absence of external Na^+ the concentration of Ca^{2+} within the cilia remains elevated for an extended period after stimulation. We therefore propose, by analogy with our earlier results from frog olfactory receptor cells (Reisert & Matthews, 1998), that a Na^+–Ca^{2+} exchanger is present in rodent olfactory cilia also (Noe *et al.* 1997) and plays a role in the extrusion of Ca^{2+} that enters through cyclic nucleotide-gated channels during the odour response (Frings *et al.* 1995; Leinders-Zufall *et al.* 1997). Nevertheless, in mouse the prolonged response in low Na^+–choline solution declined progressively, falling to 50% of its original value in 0.34 s, which is considerably faster than in the frog, where 40% of the current was still present after 5 s (Reisert, 1998). This observation suggests that additional mechanisms for Ca^{2+} removal from mouse olfactory cilia may also be present.

It is generally accepted that increases in intracellular Ca^{2+} evoke adaptation of olfactory receptor cells (for review see Menini, 1999). Such adaptation has frequently been investigated using a paired stimulus protocol (Kurahashi & Shibuya, 1990; Kurahashi & Menini, 1997; Leinders-Zufall *et al.* 1998, 1999; Reisert & Matthews, 1998; Ma *et al.* 1999). The degree of recovery that we observed in this study for a 5 s interstimulus interval under control conditions is in broad agreement with these previous reports. The ability of exposure to low Na^+–choline solution between the stimuli to impair recovery from adaptation implicates Ca^{2+}, and its extrusion by Na^+–Ca^{2+} exchange, in the control of olfactory receptor cell sensitivity in the mouse, as we have argued previously in frog (Reisert & Matthews, 1998). However, while in frog olfactory receptor cells the response to the second odour stimulus could be completely prevented by exposure to low Na^+–choline solution even after long interpulse intervals (Reisert & Matthews, 1998), such a strong suppression was not observed in mouse olfactory receptor cells. Here, even after recovery periods as short as 1 s in low Na^+–choline solution, receptor current responses could always be evoked by the second odour stimulus, even when significant currents were still present from the first response. Such recovery is presumably governed both by the Ca^{2+} sensitivity of the various adaptational mechanisms and also by the time course of the decline in the intraciliary Ca^{2+} concentration after stimulation, neither of which is yet known with any certainty.

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